Am ndments to the Sp cification:

Please add the following new paragraph at the beginning of the application on page 1:

--This application is a 35 USC § 371 National Phase Entry Application from International Application No. PCT/EP00/02607 filed on March 23, 2000, and designating the U.S.--

Please replace the paragraph at page 12, lines 21-30, with the following revised paragraph:

--The PCR product was purified using the PCR Quiaquick Purification System (QIAGEN GmbH, Max-Valmer-Strasse 4, 40724 Hilden, Germany) and ligated with Rapid Ligation Kit (Boehringer Mannheim GmbH, supra). The ligated vector was used to transfect Epicurian Coli XL1-Blue (E. coli) (Strategene, 11011 North Torrey Pines Road, La Jolla, CA 92097) by heat shock. After transfection, the cells were plated out in LB-Agar supplemented with 30 µg/ml kanamycin and incubated for 16 hours at 37°C. The DNA of some of the resulting colonies was isolated by minipreps and analyzed with restriction enzymes to confirm that it corresponded to the expected modified vector.—

Please replace the paragraph on page 11, lines 15-24, with the following revised paragraph.

--Fig. 5 shows sequencing the inserts that target GFP localization. A) The GFP fusion in clone 02/11#22 shows a strong nucleolar localization with a faint homogeneous nuclear background. B) The insert from this clone contains a well defined bipartite NLS (red) and meets the consensus of a nucleolar localization signal. C) In clone 09/07#18 GFP colocalised with the ER as shown by ~~counterstanding~~ counterstaining with an antibody against α-calnexin (not shown). D) The insert from this cell line

encodes a peptide of 35 amino acids that contains a predicted trans-membrane motif (SEQ ID NO:9 PMSIFQLIYFLLFLFLGVIC). This sequence does not have a match in the sequence databases. Scale bar = 15μm.--

Please replace the paragraph on page 15, lines 1-27, with the following revised paragraph.

--These are a few examples of the patterns of GFP localization that we have observed. Using GET we have been able to identify cells with GFP localized in every major organelle and compartment. These observations illustrate the power of GET to identify specific molecular associations to organelles and compartments. To demonstrate the use of GET to identify proteins sequences that carry targeting signals we have cloned and sequenced the DNA inserts from some of these cells. As expected, we have found sequences that correspond to known proteins and contain targeting signals which are consistent with the observed localization of the GFP fusion. One of these is clone 02/11#22, (Figure 5A, B). The GFP fusion in this cell line shows a distinct nucleolar localization with a week nuclear background. The insert from this line is identical to a fragment that spans between amino acids 62 and 131 of the mouse homologue of the HTLV-1 tax responsive element binding protein TAXREB107 (Nacken et al., Biochim Biophys Acta (1995), 1261:432-434). This fragment contains a well defined bipartite nuclear localization signal (SEQ ID NO: 10 KRKYSAAKTKVEKKKKKE) and meets the consensus of a nucleolus localization signal. We have also found inserts that re new sequences which do not have a match in the databases. This is the case of clone 09/07#18 (Figure 5C, D). These cells contain GFP that is tightly localized to the

endoplasmic riticulum (ER), as shown by counterstaining with an antibody against the ER marker α-calnexin (not shown)(Cannon et al., J. Biol. Chem. (1999), 274:7537-7544). The insert from this cell line encodes a peptide, 35 amino acids long. It does not have a match in the sequence databases, but contains a predicted transmembrane motif (SEQ ID NO:11 PMSIFIQLIYFLLFLFLGVIC) that may occur for the ER specific retention shown by the fusion protein (Dotti et al., Cell (1990), 62: 63-72.—

Please replace the paragraph which begins on page 15, line 31 and ends on page 16, line 19, with the following revised paragraph.

--Construction of the GET vector (GET#1). Using primers A (SEQ ID NO:1 CATGTTGGCGGCCGCGGTACCGTCGA) and B (SEQ ID NO: 2 GCCCGGGCGTGAGCAAGGGCGAG) we modified pEGFP-N1 (Clontech) by PCR to introduce a SrfI site between nucleotides three and four of the GFP coding sequence. This insertion shifts the initial ATG codon of the GFP out of frame with the rest of the coding sequence. This ensures that only insert-containing plasmids will express GFP. PCR was carried out with the Expand High Fidelity PCR System (Boehringer). Oligo A also introduced a Not1 site 10 nucleotides upstream of the GFP CDS. The PCR product was purified using the PCR Quiaquick Purification System (Quiagen), ligated with Rapid Ligation Kit (Boehringer) and used to transform Epicurian Coli XL1-Blue (E. coli) (Stratagene), by heat-shock. Transformed cells were plated out in the LB-Agar supplemented with 30 µg/ml kanamycin and incubated for 16 hours at 37°C. The modified vector was then isolated by minipreps and the Not1 fragment subcloned into a pQE31 vector previously modified to introduce a Not1 site between the BamHI and KpnI

sites with an adaptor made with oligos Not1-1b (SEQ ID NO:3 GATCGCGGCCGCGTAC) and Not1-8 (SEQ ID NO:4 GCGGCCGC). The resulting colonies were checked under a transiluminator to test the express of GFP and the Not1 fragment was then isolated from one of the colonies and subcloned into pEGFP-N1-Not, a modified version of pEGFP-N1 that carries an additional Not1 site inserted in position 635-642.—

Please replace the paragraph which begins on page 17, line 24 and ends on page 18, line 7, with the following revised paragraph.

--Cloning of the DNA fragments encoding subcellular localization sequences. These were isolated from cloned cells by RT-nested PCR using oligos Fir (SEQ ID NO: 5 AGCTTCGAATTCGCGGCCGCCAACATG) Sec (SEQ ID NO: 6 TATGATCTAGAGTCGCGGCCGCTTTAC) Thi (SEQ ID NO: 7 TAGCGCTACCGGACTCAGATCTCGAGC) and Fou (SEQ ID NO: 8 AAAACCTCTACAAATGTGGTATGGCTG) which flank the SrfI site of the GET#1 vector. mRNA isolation was carried out using the mRNA Capture Kit (Boehringer). The reverse transcriptase reaction and the first round of PCR were carried out using Titan One Tube RT-PCR Kit with Expand High Fidelity PCR System (Boehringer). Oligo Fou was used t prime the RT reaction. The first and second rounds of PCR used oligos Thi and Fou and Fir and Sec as primers. The PCR product was run on an agarose gel and isolated with QIAEX II Gel Extraction Kit (QIAGEN). The isolated fragment was then digested with Not 1 and subcloned into the GET#1 vector to check that the isolated fragment drives the GFP to the expected localization and for sequencing.--